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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: James A. Thomson | Date: September 26, 1997

Serial No.: 08/591,246 | Group Art Unit: 1815

Filed: January 18, 1996 | File No.: 960296.93723

For: PRIMATE EMBRYONIC STEM CELLS | Examiner: B. Brumback

AMENDMENT

Assistant Commissioner For Patents
Washington DC 20231

Dear Sir:

In response to the Office Action dated March 29, 1997 in the file of the above-identified application, please amend the application as follows:

In the Claims:

Please amend Claims 1, 3, and 9 as follows:

1. (Amended) A purified preparation of primate embryonic stem cells which (i) is capable of proliferation [in vitro] in an *in vitro* culture for over one year, (ii) maintains a [normal] karyotype in which all the chromosomes characteristic of the primate species are present and not noticeably altered through prolonged culture, (iii) maintains the potential to

differentiate [to] into derivatives of endoderm, mesoderm, and ectoderm tissues throughout the culture, and (iv) will not differentiate when cultured on a fibroblast feeder layer.

3. (Amended) A purified preparation of primate embryonic stem cells wherein the cells are negative for the SSEA-1 marker, positive for the SSEA-3 marker, positive for the SSEA-4 marker, express alkaline phosphatase activity, are pluripotent, and have [normal] karyotypes which includes the presence of all of the chromosomes characteristic of the primate species and in which none of the chromosomes are noticeably altered.

9. (Amended) A method of isolating a primate embryonic stem cell line, comprising the steps of:

(a) isolating a primate blastocyst;

(b) isolating cells from the inner cell mass of the [blastocyte] blastocyst of (a);

(c) plating the inner cell mass cells on embryonic fibroblasts, wherein inner cell mass-derived cells masses are formed;

(d) dissociating the mass into dissociated cells;

(e) replating the dissociated cells on embryonic feeder cells;

(f) selecting colonies with compact morphologies and cells with high nucleus to cytoplasm ratios and prominent nucleoli; and

(g) culturing the cells of the selected colonies.

REMARKS

By an Office Action dated March 29, 1997 in the file of the above-identified application, the Examiner in charge of this application has rejected the application on a variety of grounds. By this response and the documents accompanying herewith, the applicant responds to each of those grounds of rejection described in the Office Action.

Rejection Under 35 U.S.C. §112, second paragraph

On pages 2 and 3 of the Office Action, the Examiner objected to Claims 1-11 based on various wording informalities in the claims as filed. By amendments to the claims made above, the applicant has responded to each of those grounds of rejection.

With regard to the language of Claim 1 dealing with *in vitro* culture, the applicant has adopted the claim language suggested by the Examiner. The applicant wishes to express his gratitude to the Examiner for suggesting appropriate language that would satisfy this informality.

With regard to the use of the term "normal karyotype" in Claims 1 and 3, the language has been amended in both those claims to recite the metes and bounds of what a normal karyotype is. This definition of normal karyotype is found in the specification on page 22, lines 9 to 11.

The Examiner also pointed out an informality with regard to the phrase "differentiate to derivatives," which has been corrected as also suggested by the Examiner.

Lastly, the clerical informality in Claim 9 referring to a "blastocyte" has been corrected.

It is believed that these corrections cure all of the deficiencies pointed out by the Examiner under 35 U.S.C. §112, second paragraph.

Rejection Under 35 U.S.C. §112, first paragraph

On pages 3 through 5 of the Office Action the Examiner as imposed a rejection under 35 U.S.C. §112, first paragraph, on the grounds that the application of the present invention is not enabling without a deposit of a particular exemplary strain of the practice of the present invention. The applicant respectfully traverses this requirement and asserts that no deposit is necessary to enable the patent application of the present invention.

The applicant acknowledges that cell line designated R278.5 is an exemplary cell line for filling all the conditions of the cell preparation of the present invention. The applicant does not agree that there are no other cell lines described in the present patent application which also meet all those condition. Described in pages 27 and 28 of the application are two additional rhesus monkey cell lines designated R366 and R367 which also meet all those characteristics as defined by Claims 1 and 3. The marmoset cell line designated CJ11 also meets those characteristics.

It is submitted here that the specification of the present invention is intended, and is, sufficiently detailed in its enablement and the methods used to practice the invention such that anyone of ordinary skill in the art can reproduce a primate embryonic cell line in accordance with the methods described in the present invention. The applicant here has created four such lines and has detailed in great detail in the specification exactly how those lines were

created and how similar lines may be created. The Examiner will find in the application no claim to any single specific cell line such as R278.5. The applicant admits that if that particular cell line was claimed, a deposit might be necessary. However, what is claimed is a class of primate embryonic cell lines having defined characteristics which can be created by following the methods described in great detail in the present specification.

A deposit of biological material is never required when the application itself is fully enabling such that one of ordinary skill in the art can follow the teachings of the specification and arrive at the claimed invention. Here the claimed invention is a cell line having the characteristics defined in Claims 1 and 3. This cell line may be created by the methods described in the specification, as described in detail in the examples here. No deposit is necessary to enable one of ordinary skill in the art to make such a cell line and hence the Examiner's requirement that a deposit be made is inappropriate.

It is respectfully requested that this ground for rejection be reconsidered and withdrawn.

Double Patenting

The next ground of rejection in the application is for double patenting over the then co-pending patent application by the same inventor. The co-pending application Serial No. 08/376,327 will be abandoned and hence this ground of rejection will become moot.

Rejection Under 35 U.S.C. §102/103

On pages 6 and 7 of the Office Action the Examiner imposed a rejection to Claims 1-8 and 11 under 35 U.S.C. §102(a) as anticipated by or obvious over a publication. The publication, referred by the Examiner as "Nation/World," is a copy of a news article printed in the "Nation/World" section of the Milwaukee Journal newspaper on November 4, 1994, describing the work of the applicant here.

It is submitted by the applicant that it is inherently illogical for the Patent and Trademark Office to require this applicant to demonstrate that he practiced the subject invention before the date of the Milwaukee Journal article. The Milwaukee Journal article names the inventor here, James Thomson, as the principal investigator for the work described in the article. Since the Milwaukee Journal article describes, albeit in no detail, the results of the experiments described in this patent application, how can the publication date of the article possibly be prior to the work of the applicant here? The answer is that it cannot be prior and therefor this document cannot be prior art under 35 U.S.C. §102(a) against the claim of this patent application.

However, the applicant notes that he made this same argument in the parent case to this one and the PTO apparently refuses to accept this logic, no matter how compelling. Accordingly, in order to obviate this ground of rejection, the applicant submits herewith a declaration under 37 C.F.R. 1.131 intended to put this issue to rest. The declaration establishes practice of the invention by the named applicant here prior to November 1, 1994, and therefor, prior to the publication date of the Milwaukee Journal article. It is believed that this rejection should therefore be overcome.

Rejection of Claims 1, 7 & 8 Under 35 U.S.C. §103

The first rejection under 35 U.S.C. §103 was to Claims 1, 7 and 8 over the publication by Bongso et al in view of patents to Dyer et al. and Hogan. The Bongso et al. article discloses that the researchers there used inner cell mass cells from human blastocysts to attempt to create totipotent embryonic stem cells. The Bongso et al. article specifically reports that prior to cell isolation, the embryos were grown on passaged human fallopian tube epithelial monolayers which were used as feeder layers for the developing embryos. As the embryos developed an inner cell mass (ICM), cells from the ICM were then removed for an attempt at embryonic stem cell culture development. At the point of trying to isolate the embryonic stem cells, Bongso et al. plated the inner cell mass derived cells without feeder layers and in the presence of leukemia inhibitory factor (LIF). These procedures are analogous to those which are known to work well for the isolation of murine embryonic stem cells, and would have been the most logical procedure to choose for the isolation of primate embryonic stem cells. However, as Bongso et al. specifically reports, they were unable to obtain embryonic stem cells which could be maintained in definitely in culture. Note that the authors of the Bongso et al. paper specifically report that "after second subculture, the cells differentiated into fibroblasts or died" (page 2114, right-hand column). The first subculture was specifically reported in the paper to be 10 to 14 days (page 2112) and the second subculture was reported to be after 8 to 10 days (also page 2112). Thus the cells described in the Bongso et al. paper were specifically reported to have a lifetime as stem cells, without freezing,

of no longer than 24 days. The cell cultures created by Bongso et al. neither meets the limitations of the claims of this application nor are they of any practical utility as stem cell cultures, because of their transient nature.

The results of Bongso et al.'s experiments further demonstrates that at least some of the conditions and procedures which would allow mouse embryonic stem cell cultures to be created failed to allow the initiation of primate embryonic stem cell cultures. This results makes clear that no one in the art knew what conditions or procedures, if any, that worked with murine cultures would work to allow primate embryonic stem cells to be created. As the specification of the present invention specifically reports, the use of LIF alone fails to prevent the differentiation of rhesus embryonic stem cells, just as it seemed to fail to prevent the differentiation of the human embryonic stem cells utilized by Bongso et al. In murine cultures, embryonic fibroplasts support the undifferentiated proliferation of mouse embryonic stem cells precisely because the fibroblasts produce LIF. Since purified LIF fails to prevent differentiation in the human embryonic stem cell cultures, as described by Bongso et al., it is not at all obvious from Bongso et al. how one could successfully culture or subculture primate embryonic stem cells while preventing their differentiation into fibroblasts or other committed cell lineage.

The touchstone of the obvious inquiry in a biotechnological invention is not whether the claimed method or experiment is worth trying, but is instead whether there is a reasonable expectation of success for the method or the experiment based on the teachings in the citations. Here,

the cited documents from the art teach us that at least some of the techniques that worked with murine stem cell cultures did not work to create long term primate stem cell cultures. From the data of Bongso et al., no one of skill in the art could have reasonably predicted the long term success of the primate embryonic stem cell culture technique of the applicant here. Hence, the method used to create this culture and its product, the cultures themselves, are not made obvious by these papers.

Thus it is submitted that Bongso et al. cannot make obvious the method of the present invention. The cells of Bongso et al. simply are incapable of cultivation or proliferation in culture for the time periods achieved by the cultures of cells created by the applicant here. The method described by the applicant here, as defined by Claim 9, actually works in creating long term embryonic stem cell cultures, which the method of Bongso et al. clearly does not by their own admission.

The Examiner seeks to cure the deficiencies in the reference to Bongso et al. by combining in the rejection references by Dyer et al. and Hogan. Dyer et al. describes a culture of embryonic stem cells from varied species maintained without differentiation in the presence of a feeder layer of chicken embryonic fibroblasts. Hogan is cited for the proposition that non-mouse pluripotent stem cells can be maintained on feeder layers.

It should be noted that the cell lines created by Hogan, however they are characterized, are clearly quite different from the cell lines created by the applicant here. The Hogan cell lines originate from germ cells of embryos, the germ cells being the primordial cells of the sex cells of the

mature animal. The cell cultures created are expressly dependent on the either LIF or steel factor for the maintenance of the cells in an undifferentiated state. This is clearly not required for the cells lines of the present invention, which are expressly not dependent upon or responsive to these two factors (Specification pages 27-28).

Neither the patent to Dyer et al. nor the one to Hogan cures the deficit in the teaching of Bongso et al. Neither reference teaches how to convert the cell culture created by Bongso et al. into a long-term stem cell culture. Hogan specifically teaches that either LIF or membrane associated steel factor is a necessary component to the maintaining of the embryonic stem cell in an undifferentiated form, but the cells of the present invention require neither LIF nor steel factor. Thus, the factors reported by this combination of prior art as necessary to make a long term primate embryonic stem cell culture are not used within the method of the present invention, nor are they required by the embryonic stem cells of the present invention. Accordingly, since the technology described and claimed in this application does not make use of what the prior art says is essential, it is not obvious from that prior art that the method described herein and the cells created by this method could be made. The present method must thus be deemed non-obvious over the cited prior art.

As stated above, the applicant asserts that the paper by Bongso et al. does not render the claims of this patent application obvious, even if the Bongso et al. paper is prior art. However, the applicant also wishes to point out that the copy of the Bongso et al. paper supplied by the Examiner is stamped as received by the National Institute of Health

Library on December 8, 1994. The applicant here is filing with this response a declaration under 37 C.F.R. §1.131, to overcome the citation to the Milwaukee Journal article, establishing practice of this invention prior to Nov. 1, 1994, and that same declaration should establish the practice of this invention by the applicant before the Bongso et al. paper as well. This ground of rejection should therefore be reconsidered and withdrawn.

Rejection of claims 2-6 under 35 U.S.C. §103

The Examiner also rejected claims 1,7 and 8 over a combination of the paper to Bongso et al. combined with the published patent application of Dyer and the Hogan patent, further in view of a paper by Damajanov et al. The Damajanov et al. paper is cited to show that human cells in culture can be SSEA-1 negative, and positive for SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81.

The applicant asserts that the citation to Damajanov et al. Adds nothing significant to the rejection made against claim 1 based on the Bongso et al. paper as discussed above. The fact the human EC cultures have this combination of cell surface markers is admitted by the applicant. On page 20 of the specification is a chart showing the human EC calls do exhibit this combination of cell surface markers. It should be noted, however, that never before was it demonstrated or known that noncancerous primate embryonic stem cells would exhibit these cell surface markers similar to human EC cells. It should also be noted that ES cells are significantly more useful that EC cells, and that one object of the present invention is to overcome the limitations inherent in the use of EC cells. Note the Damajanov et el. refer to their cells

lines as a "malignant replica" of embryonic cells. If Damajanov et al. knew how to create real ES cells, why would they be content to report on a "malignant replica?" The limitation in the claims of this application to normal karyotype is intended, in part, to clearly distinguish the applicants primate ES cells from prior art EC cells cultures which were capable of creating only malignant cells of abnormal karyotype. The present invention is directed twoard true embryonic stem cells, not a malignant replica of them.

The combination of Damajanov et al. with Bongso et al., and the other references, does not make obvious that primate ES cells as defined in claim 3 could be achieved. Note again that Bongso et al. was unable to create a cell culture which was viable long term. None of the other cited papers teaches how to cure this deficiency. The applicant here has achieved what Bongso et al. and Damajanov et al. sought to achieve, but did not achieve. This is evidence of a nonobvious achievement, and that achievement is clearly claimed in claim 3 in distinguishing language. Accordingly, the claim is deemed patentable over this combination of prior art.

The applicant also wishes to note that this ground of rejection is also premised on the Bongso et al. paper which may not be prior art to this patent application.

Rejection of Claims 9-11 under 35 U.S.C. §103

The Examiner applied a separate rejection under 35 U.S.C. §103 to claims 9-11 over the Bongso et al. paper taken in view of Brown et al. Brown teaches the creation of murine stem cells by a procedure that can use a feeder layer of fibroblast cells. Note, though, that even Brown et al. suggest that

feeder layers are undesirable since they "impose restraints" on the manipulation of the embryonic stem cells.

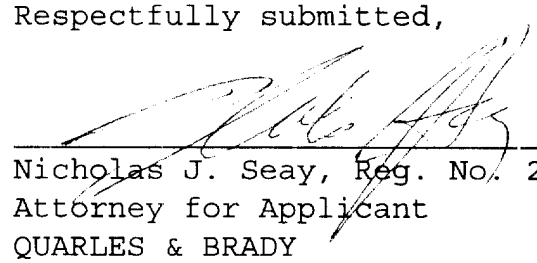
The applicants argument in response is a simple one. There are several procedures and techniques that can be used to create murine ES cells and some, but not all, of those techniques have proved adaptable to other species. The procedure used by Bongso et al., for example, does work when performed in mice, but did not make a long term viable culture in primates. The prior art does not supply guidance as to which procedures from the well developed murine systems would or would not work in primates. The Examiner's combination of Brown et al. with Bongso et al. represented the selective abstract of differing teachings from the prior art using hindsight to aid in the selection. This is inappropriate in the determination of nonobviousness under §103.

Since the prior art does not supply sufficient guidance as to which techniques or procedures developed for use with mice would work with primates, that prior art cannot make obvious the method of claims 9-11 which represent the first demonstration that true ES cells can be created in primates.

Lastly, of course, if the Bongso et al. paper is not prior art to this patent application, this rejection fails for that reason as well.

Accordingly, it is respectfully requested by the applicant that the merits of the claims of this patent application be reconsidered. An early and favorable response is solicited.

Respectfully submitted,



Nicholas J. Seay, Reg. No. 27,386
Attorney for Applicant
QUARLES & BRADY
P.O. Box 2113
Madison, WI 53701-2113

(608) 251-5000

QBMAD\142433